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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : B01L 3/00, G01N 21/03 — A1

(11) International Publication Number: WO 98/28075

(43) International Publication Date: 2 July 1998 (02.07.98)

(21) International Application Number: PCT/IB97/01585

(22) International Filing Date: 18 December 1997 (18.12.97)

(30) Priority Data:

60/033,646	20 December 1996 (20.12.96)	US
60/051,691	3 July 1997 (03.07.97)	US

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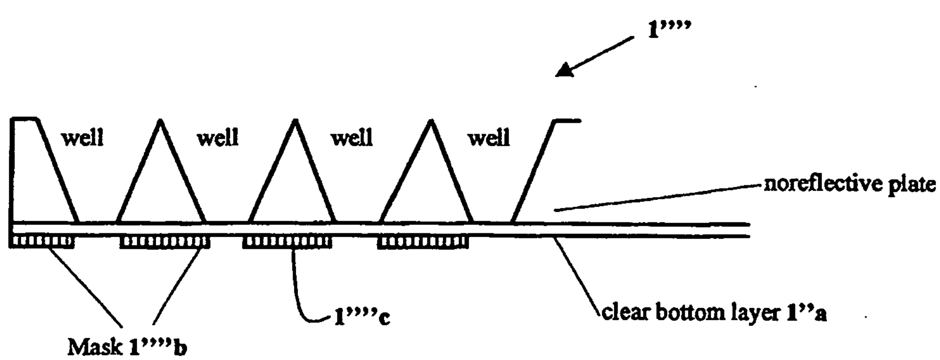
(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
With international search report.

(54) Title: A MICRO-WELL PLATE FOR IMAGING OF FLUORESCENT, CHEMILUMINESCENT, BIOLUMINESCENT, AND COLORIMETRIC ASSAYS



(57) Abstract

A specimen containment system is disclosed which optimizes the optical properties of the specimen holders (wells) for imaging in order to enhance the relevant signal, relative to background. A family of well plates all have the inherent properties of: i) minimizing reflections from an excitation source into an imaging detector; ii) minimizing the concentration of excitation into intense and localized sources (lensing); and iii) allowing an epi-illuminating excitation beam to penetrate both central and lateral wells effectively. The well plates are made of a material that has or is treated to have minimal inherent fluorescence and minimal reflection properties. The well chambers are constructed to be depth-wise asymmetric (for example, sloping well surfaces) and to have cross-sectional irregularity. The bottom walls of the wells may also be formed with a ridge or made transparent so as to minimize reflections of incident light therefrom.

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A MICRO-WELL PLATE FOR IMAGING OF FLUORESCENT, CHEMILUMINESCENT, BIOLUMINESCENT, AND COLORIMETRIC ASSAYS

Field of The Invention

The present invention concerns a device which yields improved performance in creating digital images of fluorescent specimens contained within wells or other chambers. Specifically, the invention resides in a fluid containment system ("imaging wells") that yields high performance when used with an area imaging system.

Background of The Invention

Many chemical and molecular biological assays are designed so that changes in the absorbance, transmission, or emission of light reflect reactions within the specimen. Therefore, instruments used to quantify these assays must detect alterations in luminance.

Most assays are performed within plastic plates (well plates), fabricated to contain a number of regularly spaced wells. Standard well plates contain 96 or 384 wells in an area of 8 x 12 cm. Prototype plates containing 864, 1536 or more wells are under evaluation.

Many assays use fluorescence techniques. Fluorescence measurement systems are used to quantify the amount of fluorescence emitted by samples in the wells. Standard fluorescence plate readers do this by illuminating and detecting discrete wells, singly or in small groups. Area imaging systems illuminate and detect all the wells at once.

Fluorescence assays within the wells are conducted by measuring the specific fluorescence emission of each discrete well. Fluorescence is emitted when a fluorophore (a fluorescent molecule) interacts with an incident photon (excitation). Absorption of the photon causes an electron in the fluorophore to rise from its ground state to a higher energy level. Then, the electron reverts to its original level, releasing a photon (fluorescence emission) whose wavelength depends upon the amount of energy that is released during reversion. A given fluorophore may emit at single or multiple wavelengths (creating an emission spectrum), as electrons drop from various orbitals to their ground states. The emission spectrum is constant for each species of fluorophore.

A critical aspect of fluorescence measurement is to detect the relatively faint fluorescence emission from molecules labeled with the fluorophore, while minimizing the contaminating effects of other sources of light. These other sources include the excitation illumination, nonspecific forms of fluorescence arising within the specimen and its container (e.g. fluorescent proteins or fluid around the specimen, the plate material), and optical aberrations within the detection system (e.g. scattered light within the collection optics).

The excitation wavelength is the greatest source of light contamination. It is filtered out using what is termed a "barrier" filter, placed between the specimen and the light detector. A typical barrier filter can remove about 99.99% of the excitation. However, the excitation illumination can be thousands of times as bright as the fluorescence emission. Therefore, even very good barrier filters will "leak" when specimens are illuminated so that the excitation beam shines directly into the collection optics. To prevent this, most fluorescence measurement systems use special optics to minimize detection of the excitation beam, and other irrelevant signals. Typically, epi-illumination (illumination from within the collecting lens) is used because it does not shine into the collection optics. Special fluorescence

chemistries (e.g. time resolved fluorescence) can also be used, so that the excitation beam is off during the period of detection.

With standard fluorescence measurement systems (those that do not illuminate and measure the entire plate at once), irrelevant signals are easily controlled. With area imaging systems (which illuminate and detect the entire well plate in a single operation), in contrast, irrelevant signals are more difficult to remove. One problem is that the entire plate must be illuminated evenly with intense excitation, preferably without direct entry of the excitation beam into the collection optics. Another problem is that the plate walls restrict the even entry of illumination into the wells.

An illumination and detection system has been disclosed which can yield reasonable sensitivity and accurate quantification from an area imaging system (US patent application Serial No. 60/024,043 of P. Ramm et al., the disclosure of which is incorporated herein by reference), even when used with standard well plates. Key features include a telecentric lens, epi-illumination, and a very sensitive CCD. In particular, the epi-illumination system beams the excitation away from the detector, and so minimizes the detection of the excitation wavelength.

Reasonable area imaging performance is obtainable from standard well plates. However, much better performance is available if the plates are optimized for imaging. An optimal imaging plate is quite different from a standard well plate. Most particularly, an imaging well plate needs to play a part in allowing even illumination and minimal levels of irrelevant signal over the entire plate.

The present invention is a specimen containment system which optimizes the optical properties of the specimen holders (wells) for imaging. Therefore, the present invention is a means to enhance the relevant signal, relative to background.

In one aspect, the present invention comprises a family of well plates. All of the plates have the inherent properties of: i) minimizing reflections from an excitation source into an imaging detector; ii) minimizing the concentration of excitation

into intense and localized sources (lensing); and iii) allowing an epi-illuminating excitation beam to penetrate both central and lateral wells effectively.

In addition, the present invention seeks to minimize the area of smooth, flat surface in a well plate facing the collection optics. Standard well plates usually have flat surfaces at the top and bottom of the wells. In plates with opaque bottoms, the flat bottoms reflect illumination back into the epi-illuminating collection lens and raise the level of background relative to signal. The present invention uses angled, sloping, pitted, or roughened well bottoms, which act as light diffusers or light traps. These diffusers or traps do not reflect illumination back into the collection optics.

Standard well plates have a large proportion of their surface area lying outside the wells. Typically, the wells might only occupy 60-75% of the plate. This leaves large areas of flat, reflective plastic facing the collection optics. In an epi-illuminating system, this large area of reflective background contributes significant amounts of scattered, background illumination. The present invention uses sloping well walls, which leave much less flat surface area between wells, and present a less reflective surface to the collection optics.

A fluorescence imaging system achieves the best sensitivity with an epi-illuminating optical system. Epi-illumination must be able to reach the contents of all wells evenly. However, the illuminating beam is at varying angles to the wells. Therefore, deep wells prevent uniform entry of the beam. At some locations in the plate, the beam is parallel to the well axis and will enter even a deep well without difficulty. At other locations, the beam is not parallel so that deep well walls interfere with entry of the beam. The present invention uses shallow and sloping wells, to minimize shadowing by well walls.

Standard wells have highly symmetric tubular shapes. Therefore, fluid in the wells tends to form strong meniscal lenses, which reflect or refract images of an illumination source into the detection optics (lensing effects). The lensing effect is

so bright, that it cannot be removed adequately with barrier filters prior to detection. In its preferred format, the present invention uses rectangular or polygonal wells with sloping walls. These wells are less prone to form a meniscus in the well contents, thus avoiding meniscal lenses.

Imaging well embodiments in accordance with the invention are described herein in two formats: within opaque plates and within clear-bottom plates. These imaging wells have in common the properties of: i) minimizing fluorescence signals originating in the materials of the specimen container; ii) minimizing reflections of an illumination source in the detected image; iii) minimizing lensing effects within the sample contents; iv) allowing excitation light to enter the wells with minimal shadowing by the walls. Therefore, the present invention enhances the quality of data obtained by imaging fluids in wells. Most particularly, the present invention enhances a specific fluorescence signal relative to background, and allows an improvement in the ratio of fluorescence signal to noise. In addition, the imaging wells of the present invention are suited to containing fluids for any form of luminescence or colorimetric analyses.

In accordance with the present invention the well plates are made of a material that has minimal inherent fluorescence and minimal reflection properties. Unlike standard well plates, which are fabricated from glossy or semi-glossy polystyrene or polypropylene, the present invention uses a black (or specifically colored to absorb wavelengths outside the emission range), pitted or rough medium. In one preferred configuration, the present invention is constructed from a polymer that absorbs excitation illumination, is chemically inert, and that has surface roughness (e.g. a granularity of about 10 um), microcracks, or pits. The surface roughness has two advantageous properties: 1) It diffuses incident illumination over a wider angle than smooth surfaces, so that less is reflected directly back into the collection lens; and 2) It provides more surface area of absorptive material to minimize reflections. Although this type

of material tends to absorb a portion of the emitted fluorescence, it absorbs a relatively greater proportion of the excitation illumination, preventing it from being reflected back into the collection optics.

It is a feature of the present invention that those wells constructed with opaque bottoms may have a light diffusion or light absorption mechanism in the bottom. A diffusion mechanism might be a roughened surface, or a ridge in the center of the bottom surface. An absorption mechanism might be a surface manufactured with microscopic cracks or pits. These would tend to trap light within themselves.

Another feature of the present invention is that the well chambers can be depth-wise asymmetric. For example, ridged or sloping well surfaces can make the well depth-wise asymmetric. However, it will be understood that any well shape which is asymmetric about a plane substantially perpendicular to its depth will be depth-wise asssymetric. Similarly, cross-sectional irregularity is desireable. A well with a rectangular or polygonal cross-section is more irregular than a tubular wells. Regular chambers (such as tubular wells with flat bottoms) are more prone to shape their contents into lenses. Asymmetric and irregular chambers are less prone to lensing, and depth-wise assymetric wells draw fluid to the perimeter of the well and flatten out the meniscus in the contents.

Another feature of the present invention is that the wells are shallow. In a standard well plate, the ratio of depth to width might be 3:1 or more. This design is used to maximize the fluid volume that can be held, and to minimize the fluid surface area so that evaporation is also minimized. In the present invention, the ratio of depth to width is shallow, preferably between .5:1 and 1.5:1 and most preferably about 1:1. Shallower wells have superior optical properties, in that excitation illumination is less likely to be interfered with by the well walls, and that fluorescence collection can be achieved with lenses that are not perfectly telecentric. The present invention is novel, in that it is designed to allow compromises in

fluid capacity and fluid surface area, while achieving the best area imaging performance.

The present invention is designed to minimize the reflections generated into an epi-illuminating source. In one aspect, this feature of the invention may be achieved by using wells with a clear bottom. The clear bottom may be flat, conical, or otherwise configured to yield minimal optical reflection. When epi-illuminated plates are viewed through these clear bottoms, there is minimal reflectance directly back into the collection lens.

It is also a feature of the present invention that the well walls may be sloped. Sloping walls allow the best penetration of illuminating beams into the wells. They also exhibit minimal tendency for incident illumination to reflect off the well walls back into the collection lens. Rather, the slope angle is so calculated that reflections will be directed away from the collection lens. Sloping well walls also reduce the surface area facing the illumination source.

Broadly, it is an object of the present invention to provide a well plate that minimizes extraneous signals in all forms of imaging-based assays. It is specifically contemplated that the invention provide optimal containment of liquid specimens to be imaged using fluorescence illumination.

It is an object of the invention to provide a well plate formulation that is suitable for tissue culture within the wells. The sloping walls of the invention provide relatively large surface area for cell growth within the wells, and reduced surface area (which would generate noise) outside of the wells. The use of culture-compatible plates will allow cell-based assays in wells to be conducted with high levels of sensitivity.

Although the present invention is designed to minimize reflections and other sources of contaminating illumination with specimens in wells, it is also to be appreciated that the plate formulation has benefits for specimens that are not within wells. It is an object of the invention to provide slips of the plate medium, which can serve as substitutes for the polymer membranes

used in some assays. A flat or pitted slip constructed from the plate medium may be treated to accept small volumes of fluid onto its surface. Just as the non-fluorescent and minimally reflective properties of the plate formulation are useful in imaging liquid within wells, these same properties are useful in imaging hybridization to arrays of cDNAs, oligonucleotides, or other targets applied to the plastic. The black, non-reflective material of the plate could be selected for its ability to bind DNA, or could be treated to do so.

A preferred well plate is designed to be part of a complete imaging system for high throughput assays conducted within wells. However, the present invention, by minimizing sources of noise relative to signal, could also improve performance in non-imaging detection systems. Thus, a system in accordance with the invention may be used with either an imaging or non-imaging detection system.

It is a feature of the present invention that the plates may be constructed in standard 96, 384, 864, or other well formats, with spacings between wells that are appropriate to existing liquid handling instruments.

Brief Description of The Drawings

Further objects, features and advantages of the invention will be understood more completely from the following detailed description of presently preferred, but nonetheless illustrative embodiments, with reference being had to the accompanying drawings, in which:

Figures 1a, 1b, 1c and 1d, collectively referred to below as "Fig. 1", are schematic illustrations, in side cross-sectional view, of four contiguous wells with sloping sides, with Fig. 1a showing wells with a ridged bottom, Fig. 1b showing wells with a clear bottom, Fig. 1c showing a variation of Fig. 1a in which sides of adjacent wells are minimally spaced, and Fig. 1d showing a variation of Fig. 1b in which an opaque mask is provided to cover the clear bottom;

Figure 2 is a top plan view showing a section of a well plate having wells of the type shown in Fig. 1d.

Figure 3 is a schematic illustration, in side view, of two adjacent wells in a plate, showing, at the left, how the reflections of incident light rays are directed away from the collection lens by the ridges at the bottoms of the wells, and showing at the right how the fluid within the well emits fluorescence; and

Figure 4 is a schematic illustration of the present invention, as used with an imaging system designed for analysis of assays within wells.

Detailed Description of The Preferred Embodiments

Turning now to the details of the drawings, Fig. 1 contains cross-sectional schematic diagrams illustrating embodiments of a well plate 1 in accordance with the present invention. Well plate 1 comprises a piece of black plastic, carbon fiber, or other nonreflective medium in which are cast any number of wells. Each well is constructed with a shallow form factor, preferably exhibiting a depth to width ratio in the range of .5:1 to 1.5:1, and a rectangular (see Fig. 2), or other polygonal, shape at the top. The shallow form factor allows even application of epi-illumination over the entire surface of the plate. The rectangular shape profile minimizes lensing.

Figure 1a illustrates a plate 1′ with an opaque well bottom (1′a) which is raised in a ridge 1′b so as to avoid presenting any flat surfaces to incident illumination. It will be appreciated that the bottom could otherwise be treated so as to be non-reflective (e.g. roughening).

Figure 1b illustrates a plate 1′′ in which the well bottoms are formed by a clear plate 1′′a so as to be transparent, so the ridge is not necessary.

Figure 1c illustrates a well plate 1′′′, which is similar to wall plate 1′ of Fig. 1a, except that the wells are positioned so that their sloping inside surfaces intersect at the top. This minimizes the area of the plate top surface which is

exposed to incident radiation, which would tend to reflect the same if left horizontal (in Fig. 1c).

Figure 1d illustrates a well plate 1'''' which is similar to well plate 1'' of Fig. 1b, except the wells have, again, been brought into close proximity, as in Fig. 1c, in order to have the sloping inner surfaces intersect and, furthermore, an opaque mask 1''''a is provided below the clear bottom 1''a, the mask has apertures 1''''c which coincide and are in registry with the well bottoms. This mask minimizes light reflections.

Figure 2 is a top plan view of a small section of a well plate in which the wells have the structure shown in Fig. 1d. This figure illustrates the preferred rectangular shape of the well top and bottom.

The wells of Figure 1 have sloping walls. These walls minimize shadowing of incident illumination, which tends to occur with near-vertical walls. Sloping walls also create a more asymmetric fluid chamber, which decreases lensing, and have the advantage that there is less flat surface at the top of the plate to reflect back into the collection optics.

The sloping walls also allow a more shallow form factor. The slope of the walls is calculated to produce minimum reflection from the walls back to the collection lens and will depend very much on the application. Preferably, the slope is in the range of 10-30° from vertical.

Figure 3 is a schematic diagram, showing how incident lumination rays 4 from an epifluorescent source (light comes from within the collecting lens) are directed at the specimen. At left, reflections 5 from the ridged well bottom surfaces 1'a are shown. Instead of reflecting directly back at the illumination source, most of the incident rays are reflected onto other surfaces, or laterally out of the collection angle of the lens. In contrast, the liquid within the well at right emits diffuse fluorescence over a broad angle, and is detected efficiently by the collection lens.

Figure 4 is a schematic illustration of a complete plate imaging system, as configured for epifluorescent illumination of the present invention, the system making use of well plates in

accordance with the present invention. Chromatic light from the arc lamp 10 is directed to a filter 14 lying within an optical coupler or filter wheel 16. The filter 14 removes all but the required wavelength, so that monochromatic light then travels via a fiber optic 18 to a ring light 20 mounted within the lens 22. The ring light 20 directs excitation illumination through the front of the lens, onto the plate 1. Both fluorescence emitted by specimens within the wells, and reflections of the excitation source are captured by the lens 22, and carried up to the barrier filter 24 mounted in the emission filter slot 26. This barrier filter 24 removes the excitation wavelength, leaving the emission wavelength to proceed to a CCD detector mounted within the cooled integrating camera 26. From the camera 26, image data are taken to a computer 28 where localization and quantification of the fluorescence signal is performed.

Although the detailed description describes and illustrates preferred embodiments of the present apparatus, the invention is not so limited. Modifications and variations will now appear to persons skilled in this art. For a definition of the invention reference may be had to the appended claims.

I Claim:

1. A micro-well plate for imaging of assays, said plate being of the type including a top surface and a plurality of wells extending below the top surface, each well having a bottom wall, said plate being constructed so as to, one of, absorb and minimally reflect light incident thereon.

2. The micro-well plate of Claim 1 further constructed so as to substantially reflect light emitted from the substance contained in a well.

3. The micro-well plate of Claim 1 wherein one of the top surface and interior surface of a well are one of chemically and physically treated to achieve the light handling properties.

4. The micro-well plate of Claim 3 wherein said treatment is coloring the treated surface so as to absorb or be non-reflective of incident light, while being reflective of light emitted from a substance within a well.

5. The micro-well plate of Claim 3 wherein said treatment is roughening the treated surface so as to reduce its reflection of incident light.

6. The micro-well plate of any of Claims 1-5 wherein at least one interior surface of a well slopes inwardly in a downward direction.

7. The micro-well plate of Claim 6 wherein two adjacent wells having said sloped interior surfaces are spaced so that the sloped interior surfaces converge substantially at the top surface.

8. The micro-well plate of Claim 7 wherein the bottom wall of a well is formed so as to have a ridge therein.

9. The micro-well plate of Claim 1 wherein the bottom wall of a well is formed so as to have a ridge therein.

10. The micro-well plate of Claim 1 wherein at least one interior surface of a well slopes inwardly in a downward direction.

11. The micro-well plate of Claim 10 wherein the bottom wall of a well is formed so as to have a ridge therein.

12. The micro-well plate of Claim 10 wherein two adjacent wells having said sloped interior surfaces are spaced so that the sloped interior surfaces converge substantially at the top surface.

13. The micro-well plate of Claim 12 wherein the bottom wall of a well is formed so as to have a ridge therein.

14. The micro-well plate of Claim 1 wherein two adjacent wells are provided with sloped interior surfaces and are spaced so that the sloped interior surfaces converge substantially at the top surface.

15. The micro-well plate of Claim 14 wherein the bottom wall of a well is formed so as to have a ridge therein.

16. The micro-well plate of any preceding Claim wherein a well is shaped so as to have an irregular cross-section in a plane substantially parallel to said top surface.

17. The micro-well plate of Claim 16 wherein the interior of a well has a polygonal cross-section in a plane substantially parallel to said top surface.

18. The micro-well plate of Claim 17 wherein the cross-section is rectangular.

19. The micro-well plate of any preceding Claim wherein a well is constructed so that the ratio between its width and depth is in the range of .5:1 to 1.:1.

20. The micro-well plate of Claim 1 wherein a well is shaped so as to be depth-wise assymetric.

21. The micro-well plate of any preceding Claim wherein a well is formed with a transparent bottom wall.

22. The micro-well plate according to claim 21, wherein the micro-well plate is formed with a transparent bottom surface which provides a transparent bottom wall for a group of the wells and an opaque mask is provided below said transparent bottom surface, said mask having an aperture in registry with the bottom wall of each well of said group.

Figure 1a

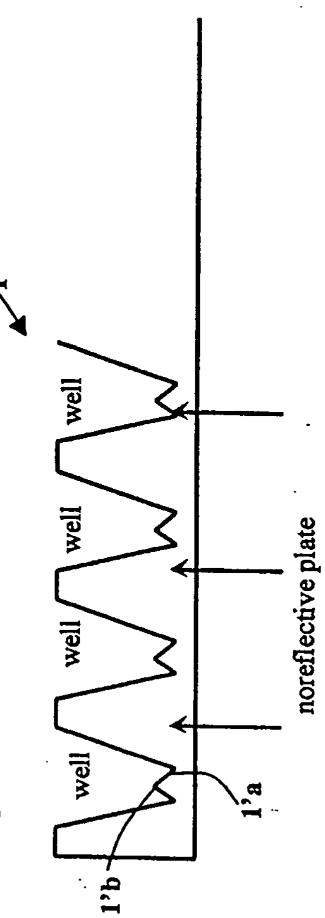


Figure 1b

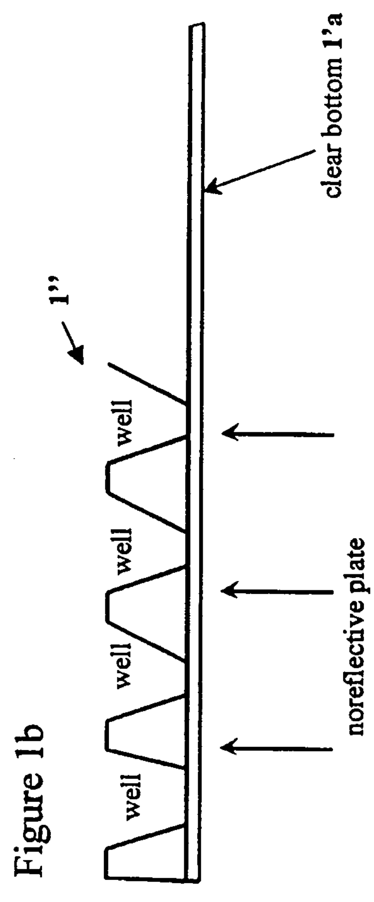


Figure 1c

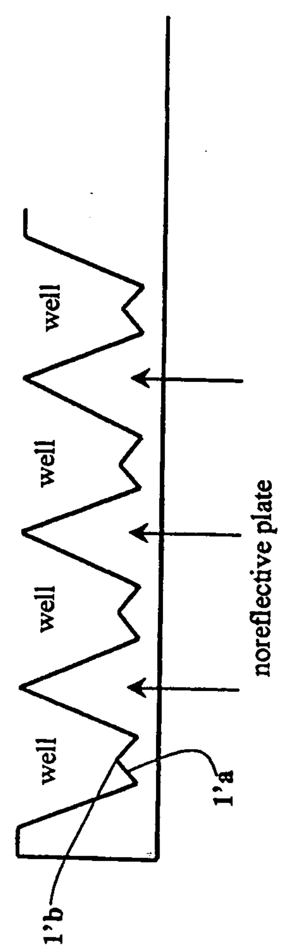
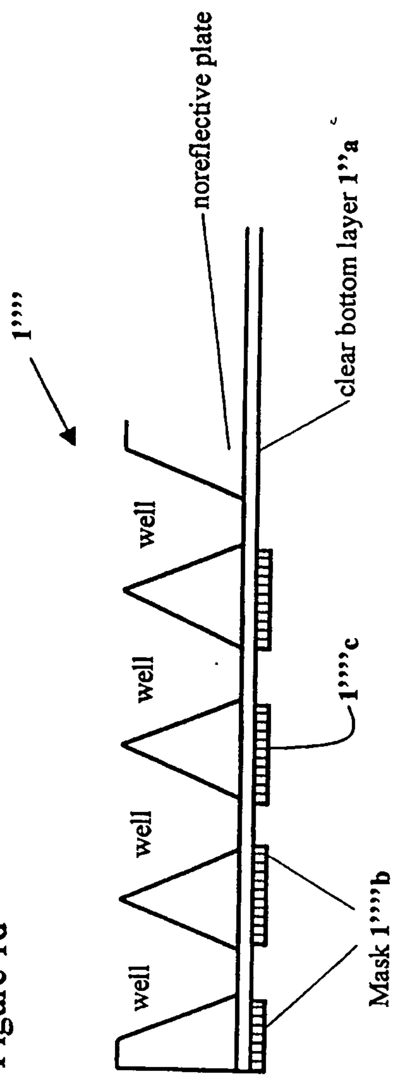


Figure 1d



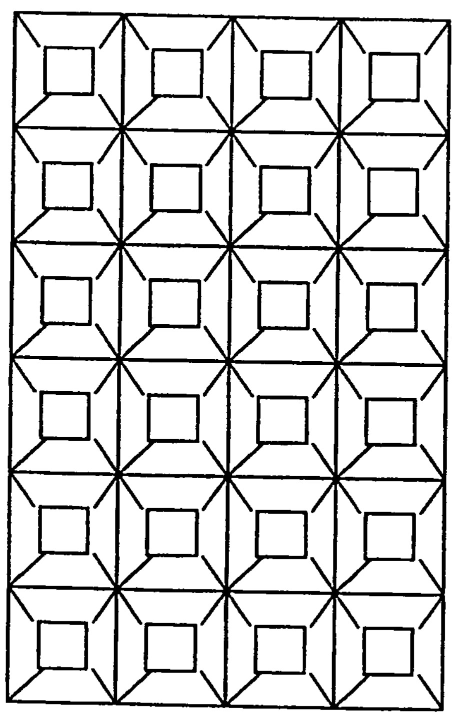


Figure 2

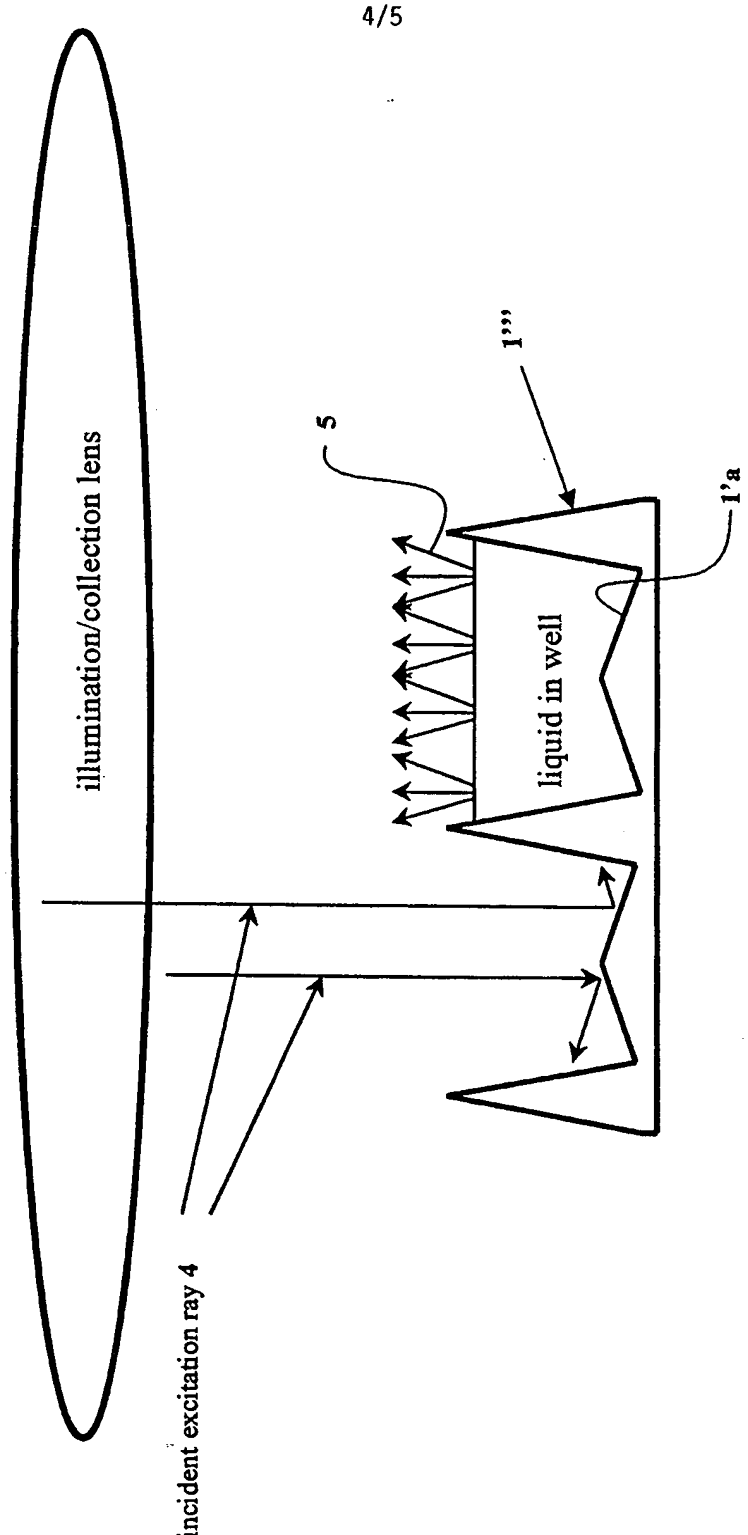

illumination/collection lens
5
1'''
1'a
liquid in well
incident excitation ray 4

Figure 3

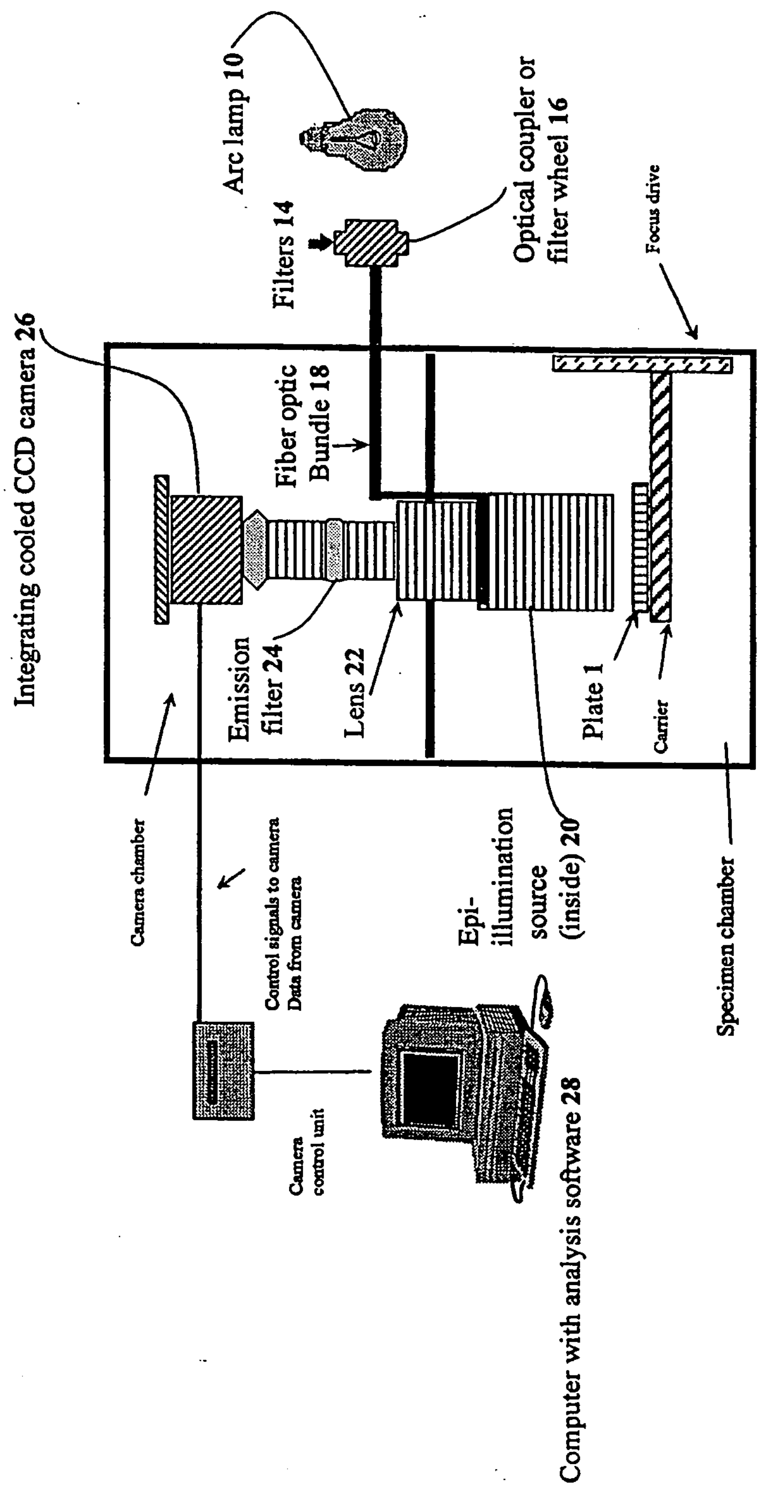

Arc lamp 10
Filters 14
Optical coupler or
filter wheel 16
Focus drive
Integrating cooled CCD camera 26
Fiber optic
Bundle 18
Emission
filter 24
Lens 22
Plate 1
Carrier
Camera chamber
Control signals to camera
Data from camera
Epi-
illumination
source
(inside) 20
Specimen chamber
Camera
control unit
Computer with analysis software 28

Figure 4

INTERNATIONAL SEARCH REPORT

International Application No
PCT/IB 97/01585

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 B01L3/00 G01N21/03

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 B01L G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 106 662 A (DYNATECH LAB) 25 April 1984 see page 11, line 5 - page 13, line 5 see page 15, line 14 - page 16, line 5 see page 16, line 21 - page 17, line 2 see page 23, line 5 - page 24, line 15 ---	1-4,6,7, 10,12, 14,19,20
A	EP 0 571 661 A (PACKARD INSTRUMENT CO INC) 1 December 1993 see column 2, line 47 - column 3, line 57 see column 5, line 1 - line 18 ---	21,22
A	US 4 240 751 A (LINNECKE CARL B ET AL) 23 December 1980 see claim 1; figure 12 --- -/--	20,21

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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"&" document member of the same patent family

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Date of the actual completion of the international search	Date of mailing of the international search report
23 March 1998	30/03/1998

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Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International Application No
PCT/IB 97/01585

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

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A	PATENT ABSTRACTS OF JAPAN vol. 095, no. 004, 31 May 1995 & JP 07 020037 A (J T SCI:KK), 24 January 1995, see abstract ---	21,22
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